# **Bone Mineral Density of the Proximal Tibia** in Wild Type and Btk Knockout Mice

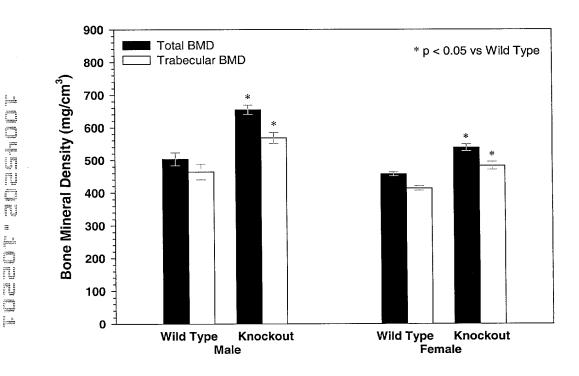


Figure 1.

Docket No.: D0032NP Applicant: C. Parick McAtee

Title: Modulators of Bruton's Tyrosine Kinase and Bruton's Tyrosine Kinase Intermediates and Methods for Their Identification and Use in the Treatment and Prevention of Osteoporosis and Related Disease States

# Bone Mineral Density of the Proximal Tibia in Wild Type and Btk<sup>xid</sup> Mice

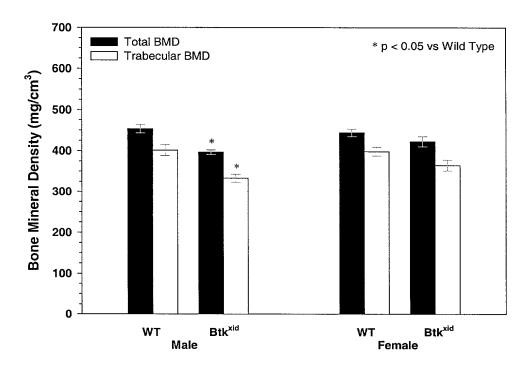


Figure 2.

Docket No.: D0032MP Applicant: C. Parick McAtee
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# Female Mice with Btklo Transgene

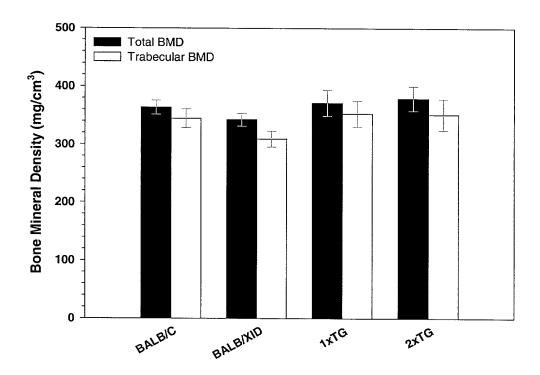


Figure 3.

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# **BTK Constructs**

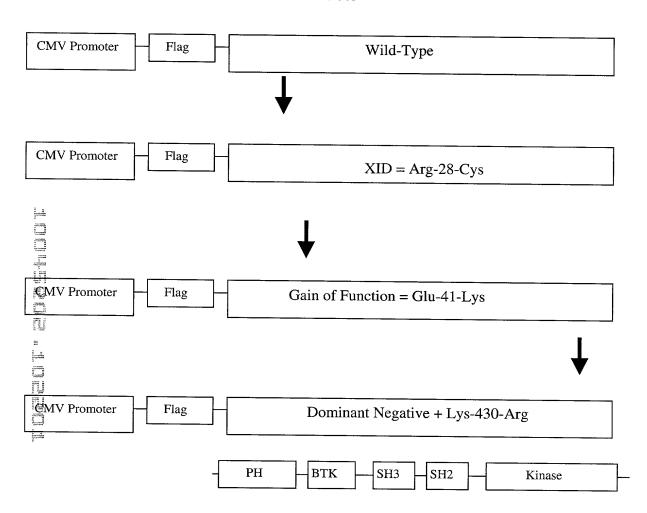


Figure 4.

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# Mu BTK wt tagged/untagged HEK/COS transient Western

**HEK 293** COS7 **HEK 293** COS7

FLAG M2 Ab

**BTK M-138 Ab** 

Legend:

Lanes 1 and 5: pcDNA

Lanes 2 and 6: BTK wt/pcDNA

Lanes 3 and 7: p3XFLAG

Lanes 4 and 8: BTK wt/p3XFLAG

Lane 9: BAP FLAG control

Figure 5.

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BTK-FLAG mutant RAW 264.7 stable mix whole cell lysates



α-FLAG Ab

Legend:

Lane 1: p3xFLAG vector control Lane 2: wt BTK in p3xFLAG Lane 3: R28C btk in p3XFLAG

Lane 4: E41K btk in p3XFLAG Lane 5: K430R btk in p3XFLAG

Figure 6.

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# **Phosphorylation Analysis of BTK Mutants**

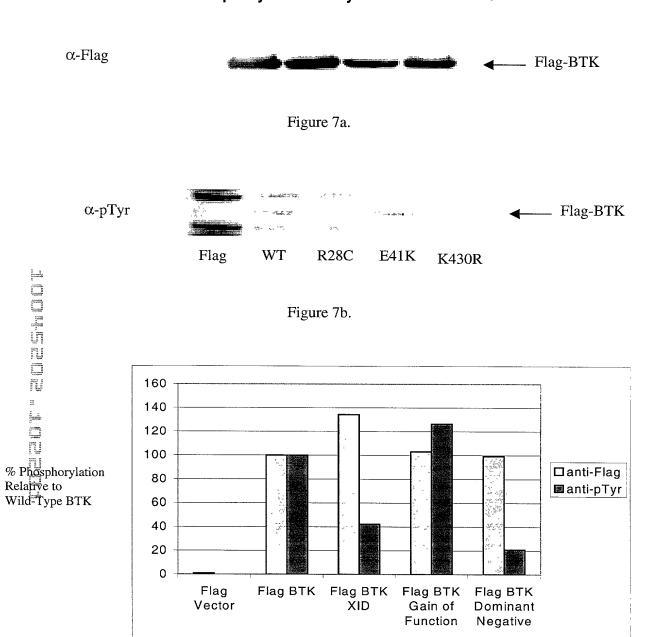


Figure 7c.

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# **Total Tyrosine Phosphorylation**

Vector WTXID  $G \ of \ F$ DN

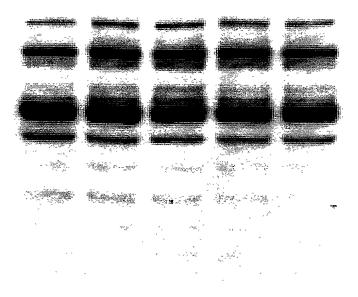


Figure 8.

Docket No.: D0032NP

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# BTK mutant immunoprecipitation kinase assays (stable pools, unstimulated)

R28C (xid Mock IP Vector Wild Type mutant) E41K K430R rBTK SLP76

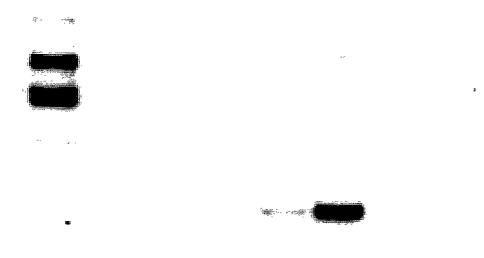


Figure 9.

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# Actin staining of Btk/RAW cell stable cell lines

# Btk wt

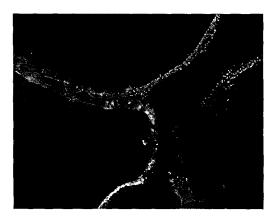


Figure 10a.

R28C-xid



Figure 10b.

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E41K-gain of function

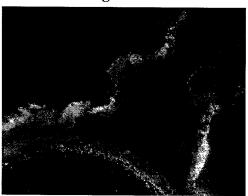


Figure 10c.